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The Association Between Vitamin D Levels and Infections in Patients with Primary Immunodeficiency

Primer İmmün Yetersizliği Olan Hastalarda D Vitamini Düzeyleri ve Enfeksiyonlar Arasındaki İlişki

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Abstract

Introduction: Vitamin D is a hormone responsible in the regulation of immune response. This study was designed to assess the relationship of vitamin D deficiency on the development of infections in patients with primary immunodeficiency (PID).

Materials and Methods: A total of 39 patients (M/F: 23/16) and 39 age-and sex-matched healthy controls (M/F: 23/16) were enrolled in the study. We measured the frequency of infections and serum vitamin D levels in winter and summer seasons.

Results: The median age of the patient and control groups were 15.0 (Interquartile range: 7.5–26.0) years and 14.0 (9.0–25.0) years, respectively (p=0.810). Winter and summer vitamin D levels were similar both in patient and control groups (p=0.492 for winter, p=0.503 for summer). Number of the patients with low serum vitamin D levels and with infections were higher in winter than in summer. Autoimmune and inflammatory diseases (AID) were predominantly observed in patients with low winter vitamin D levels and in common variable immunodeficiency (CVID) group. Patients with CVID had lower serum vitamin D levels both in summer (p=0.048) and in winter (p=0.008).

Conclusions: In this study, we showed the seasonal variation of serum vitamin D in patients with PID. There was also increased frequency of CVID and AID in those patients with low vitamin D levels. In addition, vitamin D might be given in winter to the patients with PID to take infections and autoimmune disorders under control.

Keywords: Autoimmune disorders, infection, primary immunodeficiency, vitamin D deficiency

Öz

Giriş: D vitamini, bağışıklık cevaplarının düzenlenmesinden sorumlu bir hormondur. Bu çalışma, D vitamini eksikliğinin primer immün yetersizliği olan hastalarda (PİY) enfeksiyon gelişimi ile ilişkisini değerlendirmek için tasarlanmıştır.

Gereç ve Yöntemler: Çalışmaya toplam 39 hasta (E / K: 23/16) ve 39 yaş ve cinsiyet uyumlu sağlıklı kontrol grubu (E / K: 23/16) alındı. Kış ve yaz aylarında enfeksiyon sıklığı ve serum D vitamini düzeyleri saptandı.

Bulgular: Hasta ve kontrol grubunun ortanca yaşı 15.0 (çeyrekler arası aralık: 7.5-26.0) ve 14.0 (9.0-25.0) idi (p = 0.810). Kış ve yaz D vitaminleri hem hasta hem de kontrol grubunda benzerdi (p=0.492 kış için, p0.503 yaz için). Serum D vitamini düzeyi düşük ve enfeksiyonu olan hastaların sayısı kışın yaz aylarında olduğundan daha fazla idi. Otoimmün ve enflamatuvar hastalıklar (OIH) ağırlıklı olarak, düşük kış D vitamini düzeyi olan hastalarda ve yaygın değişken immün yetersizlikli hastalık (YDİY) grubunda gözlemlendi. YDİY tanılı hastaların hem yaz (p=0.048) hem de kış (p=0.008) D vitamini düzeyleri düşüktü.

Sonuç: Bu çalışmada PİY tanılı hastalarda serum D vitamini düzeylerinin mevsimsel değişimi gösterilmiştir. Düşük D vitamini düzeyleri olan hastalarda ayrıca YDİY ve OİH sıklığı artmıştır. Ek olarak, D vitamini, enfeksiyonları ve otoimmün hastalıkları kontrol altına almak için PID'li hastalara kışın verilebilir.

Anahtar Kelimeler: Otoimmün hastalıklar, enfeksiyon, primer immün yetersizlik, D vitamini eksikliği

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Introduction

Vitamin D is one of the fat-soluble vitamins and responsible for bone metabolism also gastrointestinal absorption of minerals, mainly calcium.[1] Inactive form of vitamin D undergoes hydroxylation and is converted to 25-hydroxyvitamin D in the liver then to 1.25-dihydroxyvitamin D (calcitriol) in the kidneys. Although calcitriol is the most active vitamin D type,[1] 25-hydroxyvitamin D is the main circulating form, and measurement

of this form is therefore used widely in both clinical practice and studies.[2]

Calcitriol has many biological effects on different tissues, due to the fact that it is considered as a hormone.[3,4] Active vitamin D displays its biological functions through vitamin D receptor (VDR) located in nuclei of many cells. In recent years, VDRs have been detected in various cells of immune system.[5,6]

Vitamin D may have a regulatory function on immune system by the suppression of proinflammatory cytokines produced from monocytes and dendritic cells and by the inhibition of B cells and antibody formation.[7,8] In addition, T cells show a shift from Th1 to Th2 and regulatory T cells (Treg) via vitamin D.[9]

There are many studies demonstrating the relationship among vitamin D levels, respiratory tract infections, tuberculosis, and HIV.[10,11] However, few studies have been reported concerning with vitamin D status in patients with primary immunodeficiency (PID).[12] This study aimed to investigate the relationship among clinical characteristics, frequency of infections, and seasonal serum vitamin D levels of patients with PID on regular intravenous immunoglobulin (IVIG) therapy.

Material and Methods

Patients

This prospective clinical study was conducted between November 2016 and September 2017. The study population was consisted of two groups including 39 PID patients (Male/Female: 23/16) who received regular IVIG therapy (400 to 600 mg/kg/per month) and 39 age- and gender-matched healthy controls. The control group composed of children and adults from whom blood was drawn for routine follow-up. Definite PID diagnosis of the patients was made by using the "European Society of Immunodeficiency" (ESID) definition criteria.[13] Ethical approval was taken from the Ethics Committee of Hacettepe University *(GO 17/371–14)* in May 2016, and accordingly written informed consent was obtained from all participants or their parents.

Patients and healthy controls who were taking vitamin and mineral supplementations, steroid therapy, anti-epileptic drugs, treatment for tuberculosis, and hormone therapy were excluded from the study. There was no patient with the defect of 22q11 microdeletion or endocrine diseases related to calcium metabolism. The patient population was divided and analyzed based on the diagnosis, including humoral immunodeficiency, combined immunodeficiency, and diseases of immune dysregulation. In addition, patients were grouped according to the seasonal (winter and summer) serum vitamin D levels (deficient or normal) and the diagnosis of CVID or non-CVID.

Median time spent outdoors (hour) per day, frequency of the infections in winter and summer seasons, autoimmune manifestations, seasonal vitamin D levels, absolute neutrophil, lymphocyte, CD3, CD4, CD8, CD19, and CD16/56 counts of the patients were recorded, and all these parameters were compared between the patients with low and normal winter and summer vitamin D levels. Vitamin D levels measured only once or twice (once in summer and once in winter) and infection frequencies were recorded in healthy controls. Frequency of the infections in winter and summer seasons, seasonal vitamin D levels of the healthy group were compared to the patients.

Two invasive infections or at least three upper and/or lower respiratory tract infections within a six-month period, or necessity of intravenous antibiotic treatment for two months during a year, were accepted as recurrent infections.[14] Based on these classification, patients were classified in three groups; patients with no infection, patients who had infection once or twice, and the patients with infections ≥3 (frequent infections) during the season.

Body mass index (BMI) is defined as body mass in kilograms divided by the square of the body height in meters, and is universally expressed in kg/m^2. BMI of children was measured according to the percentiles based on age and sex [underweight (BMI was <5th percentile), normal weight (5th and 85th percentile), overweight (BMI was between 85th and 95th percentile), and obese (≥95th percentile)].[14] In adults, BMI values less than 18.5 kg/m^2 were considered as underweight, values from 18.5 kg/m^2 to 24.9 kg/m^2 were normal, values over than 25.0 kg/m^2 to less than 30.0 kg/m^2 were defined as overweight, and BMI of equal or greater than 40 kg/m^2 was obese.[15]

Measurement of Vitamin D Levels

25-hydroxyvitamin D, main circulating inactive form, was measured in all patients twice (one in summer and

one in winter period) and once or twice (one in summer, one in winter) in healthy controls during the study. Participants were classified as deficient (<20 ng/mL) or normal (≥20 ng/mL) according to vitamin D levels. [16,17] 25-hydroxyvitamin D level was measured by "high performance liquid chromatography"(HPLC) method.

Statistical Analyses

Statistical analyses were performed using SPSS Version 22.0 statistical software package (Armonk, New York, USA). Descriptive analyses were presented using median and interquartile range (IQR) for the non-normally distributed and ordinal variables. The Wilcoxon test was used to compare the changes between winter and summer vitamin D levels, and seasonal differences between the frequency of infectious diseases. To determine the significance of the variations in seasonal vitamin D levels among those three diagnosing groups, which were humoral immunodeficiency, combined immunodeficiency, and diseases of immune dysregulation, *p for trend* was calculated using the Jonckheere-Terpstra test. Bonferroni correction was applied for multiple comparisons to adjust the p value for statistical significance. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and their significance were calculated using the Spearman test. A p value of less than 0.05 was considered statistically significant.

Results

A total of 39 (M/F: 23/16) PID patients with a median age of 15.0 [Interquartile range (IQR): 7.5–26.0] years [13.0 (7.5–22.0) years for males, 16.5 (7.5–26.0) years for females] and 39 healthy age-and sex-matched controls with a median age of 14.0 (IQR: 9.0–25.0) years [14.0 (9.0–24.0) years for males, 15.9 (IQR: 8.3–32.5) years for females] were enrolled in the study (Table 1). Although there was no difference in gender, age, seasonal vitamin D levels, and presence of infections in winter and summer, only the number of the patients with frequent infections were significantly higher in the patient group than in the controls both in winter *(p=0.006)* and in summer *(p=0.043)* (Table 2).

Patients were grouped based on their diagnosis including humoral immunodeficiency, combined immunodeficiency, and diseases of immune dysregulation. Median ages were statistically different among these three diagnostic groups *(p=0.005)*. However, vitamin D levels in winter (p=0.178) and summer (p=0.028; significance was lost after Bonferroni correction) were not statistically different between those diagnosis groups (Table 1).

The median vitamin D levels of the patient group were 13.0 (IQR: 6.9–23.0) ng/mL in winter and 18.9 (IQR: 13.0–26.6) ng/mL in summer (p=0.073) (Figure 1). Although winter serum vitamin D was not different in terms of gender (p=0.350) in the patient group, summer vitamin D levels were significantly lower in female patients compared to the male patients *(p=0.002)*. Furthermore, there was no difference in seasonal vitamin D levels (p=0.403 for winter vitamin D, p=0.514 for summer vitamin D levels, Table 1, Figure 2a) between the patient and the control groups. In the patient group, winter serum vitamin D levels were low (<20 ng/mL) in 29 (74.3%) (n=13, 44.8% for females, n=16, 55.2% for males), and summer serum vitamin D levels were low in 17 patients (n=17/39, % 43.6) (n=6, 35.3% for males, n=11, 64.7% for females) *(p=0.024)*. In 13 patients (45%) of 29 those with low winter serum vitamin D levels returned to normal ranges spontaneously without any vitamin D supplements or analogous drugs in summer season. There was no statistically significant difference in time spent outdoors per day between those 13 patients with low winter serum vitamin D levels that returned to normal ranges spontaneously [4.0 (IQR: 1.0–7.5)] and the patients who had low vitamin D levels both in winter and summer [n=10, 3.5 (IQR: 0.0–5.5)] (p=0.376). In addition, absolute neutrophil, lymphocyte, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD16/56^+$ cell counts of the patients with low and normal seasonal vitamin D levels were not statistically different (Table 3).

Patients spent more time per day outdoors in summer [4.0 (IQR: 1.0–7.0) hours] than in winter season [1.0 (IQR: 0.0–3.0) hours] *(p<0.001)* (Table 4, Figure 1). And no statistical significance was determined between vitamin D levels and seasonal variation on daily spent time outdoors (p=0.424 for winter, p=0.273 for summer). The patients were divided into two groups based on spent time outdoors; at least 4 hours per day or not, and there was no statistically significant difference between these two groups neither in summer (p=0.494) and winter seasons (p=0.117). Female and male patients spent 4.5 (IQR: 1.3–5.8) and 4.0 (IQR: 1.0–7.0) hours per day in summer, respectively (p=0.703). In winter season, female patients spent a median of 1.0 (IQR: 0.0–2.0) hour outdoors per

Table 1. Clinical and demographic characteristics of the patient population

Gender (n; M/F)			23/16		
Gender (%; M/F)			58.9% / 41.1%		
Total number of the patients (n)			39		
Age of the patients (years)*			15.0 (7.5-26.0)		
Diagnosis	**n (%)**	**Age (years)* (IQR)**	**Vitamin D levels (ng/mL)* (IQR)**		
			Winter	**Summer**	***p***
Humoral immunodeficiency	**27 (69.2)**	**18.0 (12.0-26.0)**	**12.0 (5.0-20.3)**	**16.3 (11.4-22.9)**	0.239
X-linked agammaglobulinemia	5 (12.8)	19.0 (10.0-26.0)	14.6 (5.3-46.2)	30.5 (13.0-51.0)	
CVID	14 (35.9)	24.0 (14.7-32.7)	8.2 (3.6-12.2)	16.3 (6.6-20.3)	
Hyper immunoglobuline M Syndrome	3 (7.7)	19.0 (10.0-26.0)	14.6 (5.3-46.2)	30.5 (13.0-42.0)	
Hypogammaglobulinemia	5 (12.8)	6.0 (2.5-17.5)	15.5 (21.8-30.2)	18.0 (13.0-23.4)	
Combined immunodeficiency	**8 (20.5)**	**3.7 (2.2-7.5)**	**15.0 (12.2-30.4)**	**26.2 (17.6-38.4)**	0.293
SCID	5 (12.8)	3.0 (1.7-3.7)	13.6 (27.5-33.2)	27.0 (25.3-35.8)	
CD4 deficiency	1 (2.5)	13.0	14.0	18.9	
Hyper immuneglobuline E	1 (2.5)	7.0	11.7	13.9	
Ataxia Telangiectasia	1 (2.5)	7.5	13.9	56.6	
Diseases of immune dysregulation	**4 (10.2)**	**13.5 (11.0-16.7)**	**11.7 (7.2-21.3)**	**48.4 (17.6-56.3)**	0.144
ALPS	3 (7.7)	16.0 (11.0-19.5)	9.5 (7.2-13.2)	48.5 (17.6-54.3)	
CMCC	1 (2.5)	11.0	51.2	56.3	
***p* value between three Diagnosis Groups (*p* for trend)**	***<0.001***	***0.005***	0.178	0.028	
Age of onset of IVIG therapy (years)*			10.0 (5.0-12.0)		
Duration of IVIG therapy (years)*			4.0 (1.0-7.0)		
Serum IgG trough level in winter (mg/dL)*			567.5 (444.7-742.7)		
Serum IgG trough level in summer (mg/dL)*			585.0 (436.7-761.7)		
Vitamin D level in winter (ng/mL) *			13.0 (6.0-23.0)		
Vitamin D level in summer (ng/mL) *			18.9 (13.0-26.6)		
Time per day spent outdoors in winter (hours)*			1.0 (0.0-3.0)		
Time per day spent outdoors in summer (hours)*			4.0 (1.0-7.0)		

	Vitamin D levels (ng/mL)* (IQR)		
Groups based on BMI	**Winter (IQR)**	**Summer**	***p***
Underweight patients (BMI; <5th percentile) (n=12)	17.2 (6.9-30.3)	18.9 (7.1-52.3)	0.893
Patients with normal weight (BMI; 5th to 85th percentile) (n=19)	11.4 (4.9-14.0)	19.2 (13.4-27.0)	0.013
Overweight patients (BMI; 85th to 95th percentile) (n=5)	16.1 (15.5-18.3)	18.1 (13.0-21.2)	0.214
Obese patients (BMI; > 95th percentile) (n=3)	27.5 (9.0-31.2)	24.1 (23.0-30.5)	0.180
p value among BMI groups (*p* for trend)	0.954	0.933	-
Presence of autoimmune diseases (n/%)		8 (20.5%)	

ALPS, autoimmune lymphoprolipherative syndrome; BMI, body mass index; CMCC, chronic mucocutaneous candidiasis; CVID, common variable immune deficiency; F, female; IVIG, intravenous immunoglobuline, M, male; *, median (IQR: interquartile range); SCID, severe combined immunodeficiency.

day, whereas males spent 0.5 (IQR: 0.0–3.0) hour patients (p=0.810). Despite no statistically significant difference in terms of time spent outdoors per day, serum vitamin D levels of female patients were significantly lower than that of male patients *(p=0.001)* in summer. However, in the control group, vitamin D levels were similar between females [12.9 (IQR: 11.5–14.8) ng/mL] and males [16.5 (IQR: 13.8–22.4) ng/mL] in both winter (p=0.514) and summer [19.1 (IQR: 17.7–23.0) ng/mL for females, 20.3 (IQR: 18.8–23.9) ng/mL for males, p=0.403)].

Number of the patients who had at least one infection and frequent infections (≥3 times) was higher in winter than in summer (Table 4). Number of the patients with low serum vitamin D levels who had infections was higher in winter (79.3%, n=23/29) compared to that of in summer (41.1%, n=7/17) (p=0.206). However, there was no

Table 2. Characteristics of the study population

	Patients (n=39)	Healthy Controls (n=39)	*p*
Gender (M/F)	26/13	26/13	1.000
Age (years)* (IQR)	15.0 (7.5-26.0)	14.0 (9.0-25.0)	0.810
Winter Vitamin D (ng/mL)* (IQR)	13.0 (6.0-23.0)	14.0 (12.1-17.2)	0.492
Summer Vitamin D (ng/mL)* (IQR)	18.9 (13.0-26.6)	19.8 (18.7-22.8)	0.503
Number of the participants low vitamin D in winter (%)	29/39 (74.3)	16/19 (84.2)	0.403
Number of the participants low vitamin D in summer (%)	17/39 (43.6)	13/25 (52.0)	0.514
Number of the participants with infection (≥1) in winter (%)	32/39 (82)	31/39 (79.5)	0.577
Number of the participants with infection (≥1) in summer (%)	17/39 (43.5)	13/39 (33.3)	0.355
Number of the participants with frequent infections (≥3) in winter (%)	23/39 (59)	11/39 (28.2)	***0.006***
Number of the participants with frequent infections (≥3) in summer (%)	8/39 (20.5)	2/39 (5.1)	***0.043***

F, female; M, male; *, median (IQR: interquartile range).

Table 3. Relationship between seasonal vitamin D levels and immunological laboratory parameters

	Absolute counts (/mm³)	Winter vitamin D level (IQR)		*p*	Summer vitamin D level (IQR)		*p*
		Low (<20 n/mL)	Normal (≥20 ng/mL)		Low (<20 n/mL)	Normal (≥20 ng/mL)	
Neutrophil*	3700 (2800-6300)	3500 (2800-6150)	5220 (3325-6650)	0.301	3500 (2900-6600)	4350 (2700-6075)	0.944
Lymphocytes*	2000 (1500-2800)	2000 (1550-3000)	2350 (1225-2825)	0.692	2000 (1450-3000)	2000 (1475-2850)	0.900
$CD3^+$ cells*	1672 (1027-2304)	1434 (1096-2871)	1909 (968-2172)	0.956	1554 (1031-2582)	1746 (1011-2542)	0.883
$CD4^+$ cells*	810 (532-1218)	680 (520-1176)	916 (643-1643)	0.477	624 (520-1060)	963 (527.3-1806.5)	0.201
$CD8^+$ cells*	714 (507-1239)	640 (425-1254)	936 (694-1178)	0.356	640 (416-1104)	804.0 (556.5-1654.5)	0.172
$CD19^+$ cells*	210 (120-357)	369.0 (215.5-810.7)	208 (93-358.5)	0.985	221.0 (16.5-340.5)	209 (147-404)	0.568
$CD16/56^+$ cells*	93.5 (60.0-162.5)	82.0 (58.5-137.0)	156.0 (80.3-272.5)	0.215	76.0 (49.5-138.5)	118.0 (71.3-187.3)	0.265

*, Median (IQR: interquartile range).

Table 4. Vitamin D levels of the patient population in winter and summer

	Winter	Summer	p
Serum IgG level (mg/dL)*	567.5 (444.7-742.7)	585.0 (436.7-761.7)	0.227
Vitamin D level (ng/mL)*	13.0 (6.9-23.0)	18.9 (13.0-26.6)	0.073
Male	14.0 (7.2-27.5)	25.3 (20.8-40.2)	0.286
Female	11.7 (5.4-20.3)	13.9 (11.2-18.3)	0.208
Low vitamin D group	29/39 (74.3)	17/39 (43.6)	0.024
Male (n/%)	16 / 55.2	6 / 35.2	0.124
Female (n/%)	13 / 44.8	11 / 64.7	0.214
Outdoors spending time hours*	1.0 (0.0-3.0)	4.0 (1.0-7.0)	***<0.001***
Male	0.5 (0.0-3.0)	4.0 (1.0-7.0)	***<0.001***
Female	1.0 (0.0-2.0)	4.5 (1.2-5.7)	***0.001***
Infection (≥1) in patient group (%)	32/39 (82)	17/39 (43.5)	0.438
in low vitamin D patient group (%)	23/29 (79.3)	7/17 (41.1)	0.206
Infection (≥3) in patient group (%)	23/39 (59)	8/39 (20.5)	0.432
in low vitamin D patient group (%)	16/29 (55.1)	4/17 (23.5)	0.286
AID in low vitamin D patient group (%)	7/29 (24)	4/14 (23.5)	0.986
AID in normal vitamin D patient group (%)	1/10 (10)	4/22 (18.2)	0.083
Vitamin D level in patients with AID (n=8) (mg/dL)*	7.2 (3.6-11.7)	20.2 (5.4-55.8)	0.345
Vitamin D level in patients without AID (n=31) (mg/dL)*	15.5 (11.1-23.3)	18.9 (13.0-25.7)	0.132

AID, autoimmune and inflammatory diseases; IgG, immunoglubuline G; *, median (IQR: interquartile range)

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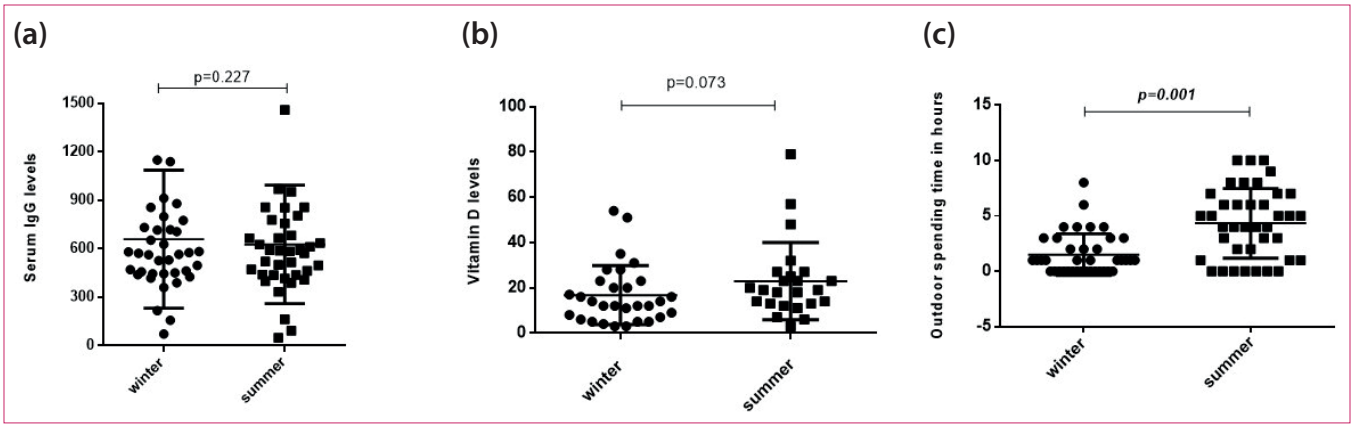

Figure 1. Differences in patients with respect to serum IgG levels (a), vitamin D levels (b), and outdoors spending time in summer and winter (c) (IgG, Immunoglubuline G).

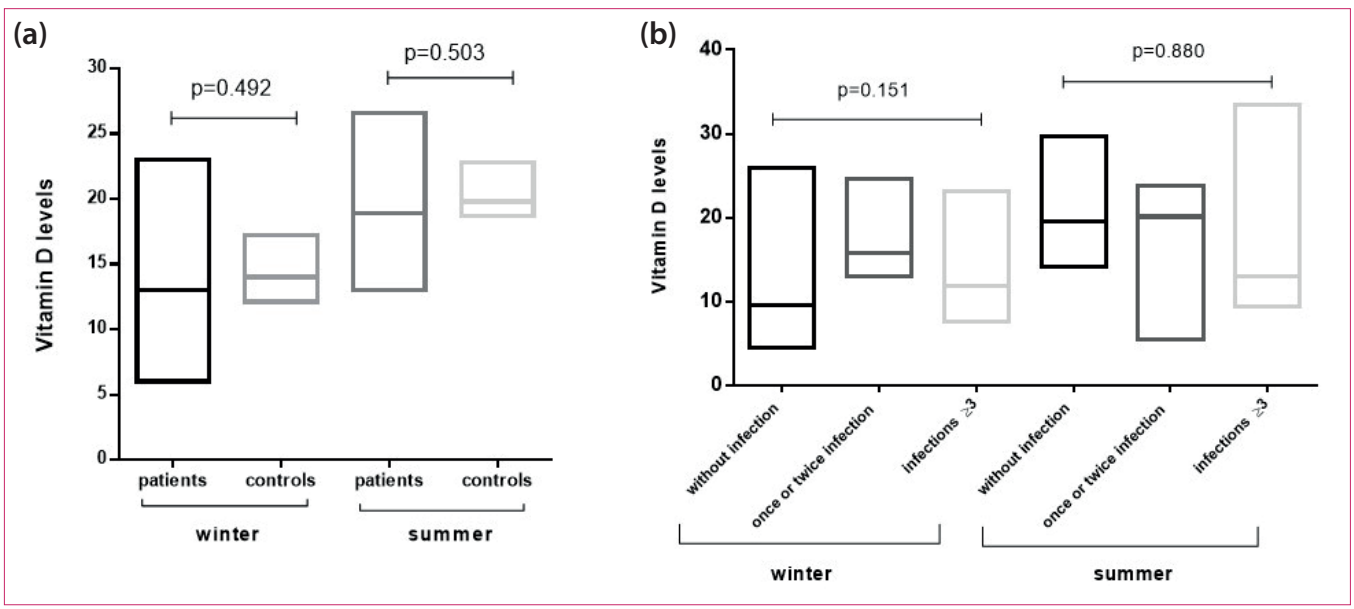

Figure 2. Differences in patient and control groups with regarding to vitamin D levels in winter and summer seasons (a). The changes in infection frequency in winter and summer in terms of vitamin D levels (b).

significant relationship between the patients with low and normal seasonal serum vitamin D levels with respect to the presence, frequency or the type of infections occurred. Patients had more frequent infections more than that of the control group in both winter *(p=0.006)* and summer *(p=0.043)* seasons (Table 2).

In winter, the median vitamin D levels was 9.6 (IQR: 4.5–25.9) for the group without infection, 15.8 (IQR: 13.0–24.6) for the group who had infection once or twice, 11.8 (IQR: 7.5–23.2) for the group with more than 2 infections (p=0.151) for the comparison of these three groups. On the other hand, in the summer season, the median vitamin D levels were 19.5 (IQR: 14.1–29.7), 20.1 (IQR: 5.4–23.8), and 13.0 (IQR: 9.4–33.4) for the groups without infection, with infection once or twice, and the patients who had more than 2 infections respectively. Likewise, there was no statistical difference among these three groups (p=0.880) (Figure 2b).

Median vitamin D level was found to be statistically significantly higher in summer in patients with normal BMI (p=0.013). However, there was no statistically significant difference in terms of vitamin D levels in the patients with higher or lower BMI (Table 1).

The patients with CVID accounted for 35.9% (n=14) of the patient group, whereas the other patients were categorized as non-CVID (n=25, 64.1%). Of the patients with the diagnosis of CVID, 78.6% (n=11) and

Table 5. Differences in parameters of the patients with the diagnosis of CVID and non-CVID

	CVID Group (n=14)	non-CVID Group (n=25)	p
Vitamin D level in winter (IQR)	8.2 (3.6-12.2) ng/mL	16.1 (11.7-28.1) ng/mL	***0.008***
Vitamin D level in summer	16.3 (6.6-20.3) ng/mL	23.2 (13.7-36.3) ng/mL	***0.048***
Patients with Low vitamin D in winter (%)	11 (78.6)	18 (72)	0.740
Patients with Low vitamin D in summer (%)	8 (57)	9 (36)	0.289
Infection (≥1) in winter (%)	13 (93)	19 (76)	0.392
in low vitamin D group (%)	10 (90.9)	13 (72.2)	0.331
Patients with Infection (≥3) in winter (%)	9 (64.2)	14 (56)	0.675
in low vitamin D group (%)	7 (63.6)	9 (50)	0.478
Patients with Infection (≥1) in summer (%)	6 (42.8)	11 (44)	0.965
in low vitamin D group (%)	3 (37.5)	4 (44.4)	0.784
Patients with Infection (≥3) in summer (%)	3 (21.4)	5 (20)	0.942
in low vitamin D group (%)	1 (12.5)	3 (33.3)	0.806
Patients with AID (%)	4 (28.5)	4 (16)	0.534

AID, autoimmune or inflammatory diseases; CVID, common variable immune deficiency

57.0% (n=8/14) had low winter and summer (<20 ng/mL) vitamin D levels, respectively (*p=0.538)* (Table 5). Serum vitamin D levels in both seasons were statistically significantly lower in the CVID group than non-CVID group (Table 5) (*p=0.008* and *p=0.048* for winter and summer, respectively). Nevertheless, no statistically significant difference was found between CVID and non-CVID groups in terms of the presence of infections in seasons (Table 5).

Autoimmune or inflammatory diseases (AID) were diagnosed in eight patients (Table 6). Demographic and clinical features of those patients with AID were given in Table 3. Serum vitamin D levels in winter were low in 7 out of 8 patients (87.5%) with AID. The median values of serum vitamin D in patients with AID (n=8) were 7.2 (3.6–11.7) ng/mL and 20.2 (5.4–55.8) ng/mL in winter and summer respectively. On the other hand, the median values of serum vitamin D of the patients without AID (n=31) were 15.5 (11.1–23.3) ng/mL in winter whereas it was 18.9 (13.0–25.7) ng/mL in summer (Table 2). Vitamin D levels were not statistically significantly different between the patients with and without AID in both winter (p=0.054) and summer (p=0.974) seasons. There were 28.5% (n=4) and 16.0% (n=4) of patients with AID in the CVID group and non-CVID groups, respectively. Furthermore, AID was diagnosed in 36.4% (n=4) of the CVID and 16.7% (n=3) of the non-CVID patients with low (<20 ng/mL) winter serum vitamin D levels (p=0.387).

Table 6. Patients with primary immunodeficiency with autoimmune or inflammatory diseases

AID	Diagnosis	Gender	Vitamin D in winter	Vitamin D in summer
RA	CVID	F	Low	Normal
DM	CVID	M	Low	Normal
DM	CVID	M	Low	Low
ITP	ALPS	F	Low	Low
ITP+AIHA	CVID	F	Low	Low
AIHA	ALPS	M	Low	Normal
Thyroiditis+IBD	ALPS	M	Low	Low
Celiac disease	XLA	M	Normal	Normal

Low, vitamin D level < 20 ng/mL; Normal, vitamin D level ≥20 ng/mL.
AID, autoimmune and inflammatory disease; AIHA, autoimmune hemolytic anemia; ALPS, autoimmune lymphoprolipherative disease; CVID, common variable immunodeficiency; DM, diabetes mellitus; IBD, inflammatory bowel disease; ITP, idiopatic trombocytopenic pupura; RA, rheumatoid artritis; XLA, X-linked agammaglobulinemia.

Discussion

Vitamin D has a role in both bone metabolism and immune system.[3,4] In the current study, serum winter vitamin D levels were low in about two thirds of the patients. This ratio is quite similar to that seen both in our healthy control group and healthy population.[18] Similarity between serum vitamin D levels in healthy population and in PID patients has been also shown in a previous report.[19] It is well-known that vitamin D levels vary according to season, gender, geographical location, and protective lifestyle.[20] In addition, we found that summer serum vitamin D levels in female patients were apparently lower than male patients despite similar time per day spent outdoors. Higher winter and summer vitamin D levels were also observed in healthy males compared to female controls, but there was no statistical significance. Vitamin D deposition in adipose tissue can cause lower serum vitamin D levels in females and obese people.[21] Therefore, serum vitamin D deficiency can be over-diagnosed in those people.[21] On the other hand, we did not find an association between BMI and vitamin D levels. Difference in summer vitamin D levels between male patients and female patients could be attributable to clothing habits and sunscreen. Clothes that cover most of the body parts for cultural reasons, and frequent application of sunscreen creams on skin in females could be the reasons of lower summer vitamin D levels in female patients with PID.[22,23]

In the study group, number of the patients with low serum vitamin D levels who had infections in winter was higher than those in summer. Although there are numerous studies showing the association between vitamin D levels and infection, reports demonstrating the effects of seasonal vitamin D variations on infections in PID patients are limited. Moreover, studies demonstrated the effect of vitamin D supplementation in diminishing the frequency of infections in PID patients.[24–26] In this study, we showed that low vitamin D levels seemed to be associated with the increased frequency of infections in PID patients only in winter season. However, we did not give vitamin D supplementation and the study was ot designed to demonstrate the impact of vitamin D supplementation on infections.

In the current study, frequency of the patients with low serum winter vitamin D levels was slightly higher in the CVID group compared to the that of in non-CVID group. There are few reports showing the relationship between CVID and serum vitamin D levels.[12,20,27] In other studies on CVID population, mean vitamin D levels were reported as 27 ng/mL ±13.33 ng/mL,[19] and 15.43 ng/mL ±7.23.[27] In addition, Baris et al found that the mean level of vitamin D in 22 CVID patients was 35.89±27.21 nmol/L (i.e.14.4±10.8 ng/mL).[28] We found that the serum vitamin D level of the CVID group was 8.2 (3.6–12.2) ng/mL in winter and 16.3 ng/mL in summer. Unlike the previous studies on PID, we measured serum vitamin D level in both winter and summer seasons. Although summer vitamin D levels were quite similar to that was reported in previous studies on CVID, winter vitamin D level was slightly lower than the values given in the reports.[19,27,28] This slight difference may be due to the geographical position of the countries where the studies have been carried out, the duration of exposure to sunlight, and the measurement of serum vitamin D levels in both seasons in our study instead of only once as seen in most of the reports.

In our study, the presence as well as the frequency of infections in the CVID group with low winter serum vitamin D levels were higher than that of the non-CVID group. In the literature, it is emphasized that CVID patients with low serum vitamin D levels have a longer duration of infections than the CVID patients with normal serum vitamin D levels.[20] Low serum vitamin D levels in CVID patients may be due to protective lifestyle and short-term sunlight exposure especially in winter season or inadequate intake of vitamin D.[29,30]

In the current study, increased frequency of AID in patients with low serum vitamin D levels in winter was shown. Agmon-Levin N et al.[31] reported lower serum vitamin D levels in patients with AID than those seen in the healthy population. Low serum vitamin D levels have been associated with an increased frequency of AID also in other reports.[32–36] However, studies published in recent years suggest that vitamin D deficiency appears to be an outcome rather than being a reason of AID.[37] In the current study, AID occurred in 28.5% of the patients with CVID. The association of AID and CVID has been reported to be 17.5% to 25% in the literature.[38–40] The variability in the frequency of AID and PID coexistence has been attributed to the underlying genetic cause of PID.[41] Higher frequency of AID in the CVID group with low serum vitamin D levels identified in the present study might be due to unfavorable effects of low vitamin D levels on AID development[35] or due to genetic characteristics of CVID.[41] A similar outcome has been reported in a previous study that serum vitamin D levels

were low in the majority of the patients with CVID and PID accompanying AID.[27] Since there are few reports on PID and vitamin D relation, further studies should be done to elucidate this complex relation.

As in all studies, there are a number of limitations related to the current report. First of all, sample size of the study population was small to compare the parameters and obtain more accurate results. Secondly, age spectrum of the patients and range of the diagnosis subgroup of PID were heterogeneous. However, this is a prospective study, and there are only few reports on PID patients and their vitamin D status. Furthermore, this is a single-center study and consisted of patients with rarely seen diseases. From this point of view, number of the study population is not too small. We also included age-and sex-matched healthy control group and made comparisons regarding with seasonal vitamin D levels and infection frequencies. For all these reason, we think that our study is valuable.

In conclusion, this study showed that higher frequency of infection and lower serum vitamin D levels were observed in winter rather than in summer season in PID patients. Although there is no difference in spending time outdoors in summer between male and female patients, summer serum vitamin D levels were significantly lower in female patients. Moreover, vitamin D levels in both seasons were significantly lower in the CVID group. The frequency of AID was also increased in the patients with low serum vitamin D levels and in the patients with CVID. This study emphasized the increased frequency of infections, AID, and CVID in the patients with low serum winter vitamin D levels, and lower summer vitamin D levels in female patients. According to the outcomes of this study, we suggest that monitoring seasonal serum vitamin D levels should be kept in mind during the follow-up of PID patients, and vitamin D may be given to the patients with PID especially in winter to take infection frequency and autoimmune disorders under control. However, more clinical studies should be done to elucidate the importance of vitamin D in patients with PID.

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